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Comparison of two sweat test systems for the diagnosis of cystic fibrosis in newborns (86/100 characters)

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Abstract (248 / 250 words)

Objectives: In the national newborn screening programme for CF in Switzerland, we compared the performance of two sweat test methods, by investigating the feasibility and diagnostic performance of the Macroduct® collection method (with chloride mesurement) and Nanoduct® test (measuring conductivity) for diagnosing CF.

Study-design: We included all newborns with a positive screening result between 2011 and 2015 who were referred to a CF-centre for sweat testing. In the CF-centre, a Macroduct and Nanoduct sweat test were performed simultaneously. If sweat test results were positive or borderline, a DNA analysis was performed. Final diagnosis was based on genetic mutations.

Results: Over five years, 445 children were screened positive and in 413 (114 with CF) at least one sweat test was performed (median age at first test, 22 days); both tests were performed in 371 children. A sweat test result was more often available with the Nanoduct compared to the Macroduct (79% vs. 60%, $p<0.001$). The Nanoduct was equally sensitive as the Macroduct in identifying newborns with CF (sensitivity 98% vs. 99%) but less specific (specificity 79% vs. 93%; p-value comparing ROC curves=0.033).

Conclusions: This national multicentre study revealed high failure rates for Macroduct and Nanoduct in newborns in real life practice. While this needs to be addressed, our results suggested that performing the Nanoduct in addition to the Macroduct might speed up the diagnostic process because it more often yields valid results with comparable diagnostic performance. The addition of the Nanoduct sweat test can therefore help to reduce the stressful time of uncertainty for parents and to start appropriate treatment earlier.

Introduction

The widespread implementation of newborn screening (NBS) for cystic fibrosis (CF) has changed the diagnostic paradigm: in contrast to patients who are diagnosed because of symptoms healthy newborns are referred for diagnostic testing after a positive screening result.[1] Apart from some newborns with meconium ileus, these children have no (or only minimal) clinical manifestation of the disease, making sweat tests the main diagnostic tool to discriminate between children with and without CF.[2-4] Sweat collection in these newborns is challenging and must be performed according to the current guidelines.[5-7] The recommendations of the Cystic Fibrosis Foundation (CFF) say that the proportion of unsuccessful sweat tests in infants should be ≤10%.[5,8] In real life, it can vary between 0 to 40% during the first three months of life.[8-12]

Determining sweat chloride concentration is the current standard criterion for the diagnosis of CF.[3,5] This is nowadays usually done with the Macroduct™ collection system that was introduced in 1986 and needs 15µl of sweat.[13-16] Measuring conductivity using the Sweat-Chek™ analyser has been suggested to be as effective as chloride determination in discriminating healthy children from those with CF.[13,17-19] Nanoduct™ is a newer sweat conductivity analysis system that was specially developed for newborns because it requires only 3–5µl of sweat and measures conductivity in situ.[20] However, only a few studies have assessed its ability to discriminate between CF patients and healthy children.[21-25] Neither American nor European guidelines have yet accepted sweat conductivity as diagnostic critera for CF.[5,6,15]

In Switzerland, CF-NBS was introduced in 2011.[26-30] With the implementation of the programme, the Swiss Federal Office of Public Health requested a close evaluation of the programme including the use and comparison of two different sweat test systems

(Macroduct™ collection system with chloride measurement and Nanoduct™ analysis system) for the diagnostic evaluation in newborns with a positive CF-NBS result. We therefore aimed to 1) compare the feasibility of the two tests in infants (overall, and according to age and weight), 2) compare the diagnostic performance of the tests in identifying infants with CF, and, 3) investigate whether the diagnostic performance of the Macroduct alone could be improved by also taking the Nanoduct result into account.

Materials and methods

The Swiss NBS programme consists of two parts: the screening part in the NBS laboratory, and, for screen positive infants, the diagnostic evaluation in the CF-centres.[28] The comparison of the two sweat test systems within the NBS was requested by the Swiss Federal Office of Public Health (FOPH) when starting the CF-NBS in 2011 and approved by the Swiss National Ethics Committee.

The Swiss CF-NBS

The Swiss CF-NBS comprises the measurement of immunoreactive trypsinogen (IRT) in a heel prick test (Guthrie card) on the 4th day of life of all newborns in Switzerland.[26-30] If the IRT is above the specified cut-off (99.2 percentile), the most common CFTR mutations (initially 7, since 2013: 18) are sought. If at least one mutation is found, the newborn is screen positive. If no mutation is found, as a safety net, a second IRT is performed if the first IRT was ≥60 ng/ml. If this IRT is again above the same cut-off the newborn is also screen positive.

This study includes all newborns screened positive and referred to one of eight paediatric CF-centres for diagnostic evaluation in the Swiss CF-NBS between January/2011 and December/2015 (**E-figure 1)**.

Diagnostic evaluation in the CF-centres

The Swiss CF-NBS uses two different sweat tests, simultaneously, one at each arm: the Macroduct™ sweat collection system (Wescor Inc., Logan, Utah, USA) followed by a coulometric determination of chloride in the laboratory and the Nanoduct™ sweat analysis system (Wescor) which measures conductivity in situ.[21] If the Macroduct and Nanoduct are positive or intermediate (or the infant had two mutations in the screening and insufficient sweat was collected or both sweat tests are negative), CFTR mutation analysis is performed

(**E-figure 1**). If both sweat tests are negative, the infant is considered a healthy carrier. If Macroduct and Nanoduct differ, the result of the Macroduct is used as decision criteria. If no Macroduct result is available, decisions on further evaluations are based on the Nanoduct. If no sweat test result is available, decision on further evaluation is based on the screening results and fecal elastase.[30] In any case, Macroduct sweat tests are repeated until a chloride result is obtained.

1. Macroduct sweat collection system and chloride determination

The Macroduct test was performed according to current guidelines; a pilocarpine iontophoretic stimulation was followed by sweat collection with the Macroduct collector system.[14,17] Sweat chloride concentration (in mmol/L) was measured by coulometry in all 8 paediatric CF centres (most used Chloridometer FGKO, Kreienbaum Neosience GmbH, 40674 Langenfeld, Germany). Sweat chloride of ≥60 mmol/L was considered diagnostic for CF, values from 30-59 mmol/L as intermediate.[3]

2. Nanoduct sweat test analysis system

The Nanoduct system induces and analyses sweat in situ while attached to the child.[20,21] Conductivity is expressed as *mmol/l eq NaCl*. This is not equal to a quantitative chloride measurement and is approximately 15-23 mmol/L higher than the sweat chloride because of additional anions such as lactate and bicarbonate.[17,18,21] A value ≥80 mmol/L was considered consistent with the diagnosis of CF, values from 50-79 mmol/L as intermediate.[13,17,21,23] In healthy newborns at the age of 3-4 weeks, mean conductivity was 36 mmol/L with a range of 12-64.[31]

3. CFTR mutation analysis

Genomic DNA was extracted from peripheral blood cells. In a first step, the laboratory tested for 50 mutations using a Multiplex-PCR and Amplification Refractory Mutation System (ARMS™; ELUCIGENE® CFEU2v1 Kit).[26,27] When fewer than 2 mutations were detected,

the entire coding sequence of the CFTR gene was screened, including intron/exon boundaries, promoter region, and tests for deletions and duplications.

Definition of final diagnosis

For the purpose of this study, we defined the final diagnosis (the "criterion standard") solely on the genetic mutations according to the CFTR2 (www.cftr2.org) or CFTR1 (http://www.genet.sickkids.on.ca/app) database or the American College of Medical Genetics and Genomics (ACMG)-Criteria[32]: a CF diagnosis was made if two CF-causing mutations were present, a Cystic Fibrosis Screen Positive, Inconclusive Diagnosis (CFSPID) if two CFTR mutations were present and at least one of them was not CF-causing (all newborns in our study that received the full genetic workup had two mutations identified).[33] We then assessed the diagnostic performance of the two sweat tests by comparing them to this diagnostic criterion standard.

To calculate the true negative and false negative sweat test results we needed to be sure that none of the children with a negative sweat test result had in fact CF. To ensure this, all children (born 2011-2015) diagnosed with CF based on clinical symptoms (outside the CF-NBS) are reported to the central database by the clinicians of the CF-centres.

Data collection

All positively screened children are registered in a central database. Clinical data, diagnostic test results and genetic mutations are reported by the physicians. This analysis used the following data: date of birth, sex, birth institution, birth weight, gestational age, CF-centre, final diagnosis, CFTR mutations and sweat test results including number of tests attempted, number of successful tests, age and weight at each test, and chloride and conductivity results.

Statistical analysis

We included all sweat tests performed at an age ≤90 days. To assess the feasibility of the tests, we used the first attempted sweat test. For the other analyses we used the first successful sweat test result (i.e. the first sweat test with a usable result). Macroduct and Nanoduct results are described separately. A sweat test was considered successful if judged as such by the performing clinician/technician and a sufficient amount of sweat was collected (quantity not successful (QNS) = <15µl for Macroduct and <1g/m^2/min for Nanoduct (shown on the display)).

We compared the proportion of successful tests (feasibility) at first attempt, overall and stratified by child age and weight. Using logistic regression we determined whether the child's age or weight was predictive for the sweat test success.

We calculated the following screening parameters for the Macroduct and Nanoduct to compare the diagnostic performance of the tests in identifying children with and without CF: sensitivity and specificity, false negative rate, false positive rate, positive predictive value, and negative predictive value. We calculated these parameters for a intermediate chloride cut-off of 30 mmol/L (Macroduct) and a conductivity cut-off of 50 mmol/L (Nanoduct), the defined cut-offs of the Swiss CF-NBS, which determine whether a child is further evaluated with genetic analysis or released as healthy[28]. We compared the areas under the receiver operating characteristic (ROC) curves to test for a significant difference of the sensitivity and specificity between the Macroduct and Nanoduct sweat test. Further, for children with both Macroduct and Nanoduct data, we plotted chloride versus conductivity in relation to the final diagnosis, and did Bland-Altman and Bias plots. These two plots allow the identification of any systematic differences between the two sweat test systems across the range of chloride/conductivity levels.

We investigated whether the diagnostic performance of the Macroduct alone could be improved by taking into account the Nanoduct result. For this, we considered two scenarios:

following up all children who 1) had either a positive Macroduct (chloride ≥60 mmol/L) or Nanoduct (conductivity ≥80 mmol/L), or 2) had either a intermediate Macroduct (chloride ≥30 mmol/L) or Nanoduct (conductivity ≥50 mmol/L).

All analyses were performed in STATA, version 14 (StataCorp LP, College Station, Texas, USA) and a p-value of <0.05 was considered statistically significant.

Results

Characteristics of study population

Over five years, 445 infants were screened positive and referred to a CF-centre for diagnostics (**E-figure 2**). Among these, 432 came to the CF-centre and 413 (50% boys, 91% born in a hospital, **Table 1**) had at least one sweat test performed by age ≤90 days. Both tests were attempted in 371 infants, and for 229 both yielded a usuable result. CF was diagnosed in 114 infants (28% of 413), 16 (4%) had an inconclusive diagnosis (CFSPID), and 283 (69%) were classified as healthy (**Table 1**). Overall, we performed 924 sweat tests: 458 Macroduct tests in 382 infants and 466 Nanoduct tests in 402. On average, infants were 22 days old (range 4–90 days) at the time of the first sweat test and weighed 3745g (range 2350–6830g).

Feasibility of Macroduct and Nanoduct

Overall, a Macroduct test was attempted in 382 infants and successful in 229 (60%, **Table 2**). Proportions of successful tests ranged from 47% to 83% in the CF-centres (**Figure 1A**). A Nanoduct test was attempted in 402 infants and successful in 317 (79%, range between centres, 57%–91%). The main reason for unsuccessful tests in both systems was an insufficient amount of sweat. The Macroduct failed significantly more often than the Nanoduct. This was true overall and within weight categories (all $p<0.001$; **Figure 1B**). Among the 149 infants (40%) with unsuccessful Macroduct tests at first attempt, 89 had a valid Nanoduct test result. Of these, 29 had CF and were correctly identified by the Nanoduct with conductivity ≥80 mmol/L. Among the 78 infants (21%) with an unsuccessful Nanoduct test at first attempt, 18 had successful Macroduct results. Of these, 5 had CF and were correctly identified by the Macroduct with chloride ≥60 mmol/L.

The proportion of successful tests increased with increasing weight ($p<0.001$ for both tests; **Figure 1B**). Age was associated with test success in the univariable analysis but only

weight remained an independent predictor in the adjusted model with an odds ratio (OR) of 3.0 (95% confidence interval [CI] 1.9–4.7) per kg increase in weight for the Macroduct, and an OR=3.5 (95% CI 2.1–6.0) per kg for the Nanoduct.

Diagnostic performance of Macroduct compared to Nanoduct

Overall, both sweat test systems discriminated well between infants with and without CF (**E-table 1**). However, within the CF patients there was one infant with a normal chloride level and two infants with normal conductivity levels; all three had 2 CF-causing mutations and were pancreatic insufficient. One healthy infant had a conductivity ≥80 mmol/L.

The clinical sensitivity of the Macroduct test system (for the intermediate cut-off of chloride ≥30) was 99%, and it was 98% for the Nanoduct system (for the intermediate cut-off of conductivity ≥50; **Table 3**); the clinical specificity of each was 93% and 79%, respectively (p-value comparing ROC curves=0.033). The positive predictive values of Macroduct and Nanoduct were 84% and 62%, respectively.

The scatterplot comparing chloride and conductivity results for the same infant (n=229) resulted in an estimated linear regression line with an intercept of 29.4 mmol/L and a slope of 0.78 (95% CI 0.73–0.83, **Figure 2**). The Bland-Altman plot showed that conductivity was on average 22.0 mmol/L higher than the chloride concentration. However, the difference decreased with increasing mean chloride/conductivity levels with a slope of -0.13 for the estimated linear regression line (95%CI -0.19 – -0.07; **Figure 3**). The same was true for the Bias plot comparing the difference between conductivity and chloride to the chloride level (slope of estimated linear regression -0.22 [95%CI -0.27 – -0.16]; **E-figure 3**).

Diagnostic performance of Macroduct and Nanoduct together

We investigated whether the diagnostic performance of the current criterion standard Macroduct could be improved by the Nanoduct results (**Table 3**). First of all, more infants had

a valid test result when considering both tests (n=371, compared to 258 for only the Macroduct). Had we followed-up every child with a positive Macroduct or Nanoduct test result (Macroduct CF positive cut-off of chloride ≥60 or Nanoduct CF positive cut-off of conductivity ≥80), the sensitivity of the sweat test would have decreased from 99% for the Macroduct alone to 92%. However, with only 1 false positive child the specificity would have improved to almost 100% (compared to 93% of the Macroduct alone). On the other hand, had we had followed-up every child with a intermediate Macroduct or Nanoduct (Macroduct intermediate cut-off of chloride ≥30 or Nanoduct intermediate cut-off of conductivity ≥50) the sensitivity would have increased to 99% (compared to 98.5% for the Macroduct alone), but so would the number of false positive test results (to yield a specificity of 78%).

Discussion

This study, done in a real-life context of a national newborn screening programme found that only 60% of Macroduct tests were successful at first attempt, with considerable variation between centres. The Nanoduct was more often successful (79%) and as sensitive as the Macroduct in identifying newborns with CF (sensitivity 98% vs. 99%, respectively), but less specific (specificity 79% vs. 93%). Considering the Nanoduct result in addition to the Macroduct alone could not improve "the Swiss" sensitivity/specificity of the diagnostics, however, 29 children with an unsuccessful Macroduct at first attempt could be correctly identified as having a CF on the basis of genotype analysis, directed by a positive Nanoduct result ≥80 mmol/L.

Strengths and limitations

This is a prospective, population-based, long-term study that closely evaluated the Swiss CF-NBS programme since its beginning in January 2011. The study reflects the daily clinical practice including the eight Swiss paediatric CF-centres and other relevant partners (national NBS and genetic laboratories). Within the study, we collect a variety of variables in a central database that included a large cohort of 432 children. All centres have indicated that they have performed the sweat tests according to current guidelines, but checking all procedures of each centre during a sweat test symposium in 2016 (after the study period) revealed variations in procedure and materials between CF-centres, which reflects findings of a recent European survey of real life practice of sweat testing.[34]

Comparison with other studies

The collection of a sufficient amount of sweat to measure chloride in infants is challenging, and studies report between 0% and 40% invalid Macroduct tests in children <3 months of age.[8-11] In our study test success increased with weight, and was higher for Nanoduct than for

Macroduct, which is in line with our previous single-centre[21] and multicentre[22] studies, and studies from other groups.[24,35] However, the proportion of unsucessful tests, 40% for the Macroduct (range between centres 17–53%) and 21% for the Nanoduct (range between centres 9–43%), was higher than in our previous and international studies. In our previous studies we had 15% and 18% of unsucessfull Macroduct tests and 3% and 6% of unsuccessful Nanoduct tests. In the Dutch study by Vernooij-van Langen et al., the proportion of unsucessful results was 7.5% for Nanoduct, and 22% for Macroduct.[24] One reason for these differences might be that he Swiss CF-NBS assesses newborns earlier and with lower weight than other studies. Furthermore we do each test only once (Macroduct on one arm and Nanoduct on the other arm) whereas others perform the same test twice in parallel. Lastly, ours was a nationwide study including all CF-centres with differently experienced staff and different methods.

For our chloride cut-off of 30 mmol/L, we had a clinical sensitivity of 99%, specificity of 93%, and positive predictive value (PPV) of 84%. The only study using a similar cut-off (34 mmol) was the Polish study by Sands and colleagues including 487 infants (45 with CF) over 3 years (2006–2009).[23] For their cut-off, they reported a Macroduct sensitivity of 100%, a specificity of 98%, and a PPV of 80%. For our conductivity cut-off of 50 mmol/L, we calculated a sensitivity of 98%, specificity of 79%, and a PPV of 62%. The study by Sands et al. with the same cut-off, reported a Nanoduct sensitivity of 100%, a specificity of 98% and a PPV of 79%. However, these two studies are difficult to compare. For the current analysis, we have explicitly used the CFTR genotype interpreted by the CFTR2/CFTR1 databases and the ACMG criteria as standard for the final diagnosis. Only this approach allows calculating the independent performance of the sweat test in discriminating CF patients from healthy individuals. The Polish study by Sands has, however, included the sweat test result in addition

to genetic mutation in the definition of their final diagnosis which will increase the sensitivity and specificity of the test.[36]

Interpretation of results and clinical implications

The proportion of unsuccessful sweat tests in the Swiss CF-NBS is too high and we are striving to improve this. Small differences in the conduct of the sweat test across centres might be a reason for the high proportion of unsuccessful sweat tests. As a result of this study, we have tried to find out reasons for the high proportion of unsuccessful sweat tests and found out that one centre did not properly clean the skin before the sweat test, and another centre has sent the Macroduct collector to the laboratory without proper sealing. A few centres have collected the sweat for more than 30 minutes, and one centre had a not properly working induction apparatus. This emphasizes the importance to pay attention to the technical details of sweat testing and train staff to exactly follow the official guidelines when performing sweat tests.[5-7] Because of these results, the PI of the study [JB] now visited different CF-centres in Europe with better sweat test results to identify differences between their and the Swiss procedures. We organized a workshop for all the Swiss paediatric CF centres to bring this expertise to Switzerland and discuss the procedures in Swiss centres, particularly in centres with a low proportion of successful tests. We will closely observe whether this initiative will improve performance or further actions need to be taken, for example reducing the number of national diagnostic centres so that the staff is more experienced. For a small country like Switzerland (8.2 million inhabitants), eight CF-centres are rather many resulting in only a few newborns tested per year in the smaller centres.

Overall, we had three false negative sweat test results, one with Macroduct and two with Nanoduct. A chloride measurement of 10 mmol/L in a child with CF and pancreatic insufficient is physiologically not possible and must be a technical failure (e.g. incorrect cleaning of the skin before sweat collection or dilution in the laboratory).[37] The two Nanoduct

results of 49 and 47 mmol/L in children with CF are most likely due to a technical problem as the conductivity level should be higher than the according chloride value of these children (77 and 89 mmol/L, respectively). We could not determine the reason for the only false positive Nanoduct result of 80 mmol/L in a healthy carrier.

All studies comparing the Nanoduct and Macroduct sweat test systems (including ours), found that the Nanoduct yields a higher proportion of successful tests in newborns.[23,24] We found that a Nanoduct result was obtained in 89 infants in whom no Macroduct result could be obtained. Among these, 29 had CF and were correctly identified with conductivity ≥80 mmol/L. Thanks to the available Nanoduct result, a presumptive diagnosis could be made in these children and appropriate CF treatment was started.[38] This is important to reduce the stressful time of uncertainty in parents awaiting a final diagnosis.[29] In the Swiss NBS, at the first visit in the CF-centre we therefore recommend simultaneously performing a Macroduct and a Nanoduct sweat test to increase the probability of at least one successful sweat test result. In any case, it is necessary at some stage to confirm the diagnosis with a sweat chloride measurement. This is important because sweat chloride is a main outcome in studies with CFTR-modulators and the only diagnostic measure for CF accepted by current American and European guidelines.[5,6,39] However, we cannot say whether performing Macroduct and Nanoduct simultaneously, one at each arm, yields more successful tests than performing two Macroducts simultaneously. This needs to be investigated in a randomized controlled trial.

We also looked at whether we could improve the clinical sensitivity and specificity of the current criterion standard Macroduct by taking into account the results of the Nanoduct test as well. We found that this approach could reduce the number of false positives, but only at the cost of a reduced sensitivity.

Conclusion

The Nanoduct more often yields a successful result due to its lower sweat weight requirement. In the presence of high Macroduct failure rates, we therefore suggest performing the Nanoduct sweat test in addition to the Macroduct for the diagnostic evaluation within the CF-NBS. This is especially relevant in very young newborns with a low weight, where there is a high probability of not getting enough sweat for a chloride measurement. The Nanoduct can add to the diagnostic matrix when sweat collection for the Macroduct is insufficient. This can hasten the diagnosis, which is important to start appropriate treatment as early as possible and reduce the stressful time of uncertainty for parents.

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Author contributions

Conceived and designed the study: CSR, CEK, JB; conducted the study: CSR, CEK, JB, MJ, SG; analyzed the data: CSR; contributed patients: AJ, CC, JB; wrote the paper: CSR, CEK, JB, MJ, SG, AJ, CC; approved the final draft of the manuscript: CSR, CEK, JB, MJ, SG, AJ, CC.

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Conflict of interest statement

There are no conflicts of interest for any of the authors.

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Table 1. Characteristics of subjects included in the study (N=413).

	n	%[a]
Child's demographic characteristics		
Year of birth		
2011	80	19.4
2012	77	18.6
2013	83	20.1
2014	101	24.5
2015	72	17.4
Sex		
Male	206	49.4
Female	204	49.9
Missing	3	0.8
Clinical characteristics		
Birth institution		
Hospital	373	90.3
Birthing centre	3	0.7
Home	25	6.1
Other	9	2.2
Missing	3	0.7
Final diagnosis		
No CF	283	68.5
CF	114	27.6
CFSPID	16	3.9
Sweat tests		
Number of Macroduct tests performed		
No test	31	7.5
1 test	315	76.3
2 tests	60	14.5
3 tests	5	1.2
4 tests	2	0.5
Number of Nanoduct tests performed		
No test	11	2.7
1 test	346	83.8
2 tests	50	12.1
3 tests	4	1.0
4 tests	2	0.5
Continuous variables		
	Median	**Range**
Gestational age (w)	39	27-49
Birth weight (g)	3280	480-4660
Age at the first visit in the CF-centre (d)	22	4-90
Weight at the first visit in the CF-centre (g)	3745	2350-6830

NOTE: Percentages are based upon available data for each variable.

Abbreviations: CF, cystic fibrosis; CFSPID, cystic fibrosis screen positive inconclusive diagnosis; d, days; g, grams; n, number; w, weeks.

[a] Column percentages.

Table 2. Proportion of successful sweat tests at the first attempt and mean weight at test for Macroduct and Nanoduct

	Macroduct		Nanoduct		p-value[a]
Proportion of successful sweat tests					
	N	% (95% CI)	N	% (95% CI)	
All tests attempted	**382**		**402**		
Tests valid	229	60 (55 - 65)	317	79 (75 - 83)	n.a.[b]
Reason tests not valid					
Not enough sweat	136	89	67	79	n.a.[b]
Technical problems	1	1	4	5	
Reason unknown	16	10	14	16	
Test pairs	**371**		**371**		
Tests valid	222	60 (55 - 65)	293	79 (75 - 83)	<0.001

Abbreviations: CF, cystic fibrosis; CI, confidence interval; n.a., not applicable.

[a] p-value from chi square statistics and t-test comparing the Macroduct and Nanoduct.

[b] p-value not applicable because the Macroduct and Nanoduct results cover different groups of children (depending whether or not the respective test was attempted).

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Table 3. Diagnostic performance for CF[a] of the Macroduct and Nanoduct sweat test and a combination of both tests.

	True positives	False negatives	False positives	True negatives	Total tests	Sensitivity	Specificity	PPV	NPV
MACRODUCT (intermediate cut-off **chloride 30-59**)[b]	67	1	13	177	258[c]	99%	93%	84%	99%
NANODUCT (intermediate cut-off **conductivity 50-79**)[b]	87	2	54	199	342[d]	98%	79%	62%	99%
MACRODUCT CF positive cut-off **chloride ≥60 OR** NANODUCT CF positive cut-off **conductivity ≥80**	89	8	1	273	371[e]	92%	100%	99%	97%
MACRODUCT intermediate cut-off **chloride ≥30 OR** NANODUCT intermediate cut-off **conductivity ≥50**	96	1	61	213	371[e]	99%	78%	61%	100%

Abbreviations: CF, cystic fibrosis; NPV, negative predictive value; PPV, positive predictive value.

[a] The final diagnosis was based on the CFTR2 database (http://www.cftr2.org/) at Johns Hopkins University, CFTR1 (http://www.genet.sickkids.on.ca/app) database at the Hospital for Sick Children in Toronto or the American College of Medical Genetics and Genomics (ACMG)-Criteria.

[b] Macroduct chloride of 30mmol/L and Nanoduct conductivity of 50mmol/L are the relevant cut-offs in the Swiss CF-NBS whether a child will be further followed up with genetic analysis and assessment of pancreatic function, or declared as healthy.

[c] Overall, we had 262 valid Macroduct tests, but only 258 with information on chloride. For four infants, unfortunately only the osmolarity was provided from the Macroduct sweat test instead of the chloride results.

[d] Overall, we had 342 valid Nanoduct tests and conductivity results.

[e] Overall, 371 children had at least one valid sweat test result.

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Figure legends

Figure 1. Proportion of succesful Macroduct and Nanoduct sweat tests by body weight and CF-centre. Fig 1 shows the proportions and 95% confidence intervals of successful Macroduct and Nanoduct sweat tests stratified by the testing CF-centre (1A) and body weight of the child [in grams] at the time of the testing (1B). The number at the bottom of each bar represents the number of children in each cell. The proportion of successful tests increased with increasing weight of the child ($p<0.001$ for both tests from univariable logistic regression models). Abbreviations: CF, cystic fibrosis; g, grams.

Legend: ■ Macroduct □ Nanoduct

Figure 2. Scatterplot comparing Macroduct and Nanoduct test result, by final diagnosis (n=229). Fig 2 shows the respective Macroduct and Nanoduct result for each child with a successful test result in both sweat tests (n=299 test pairs). The first successful sweat test was considered. With the exception of 24 children, all test pairs were performed at the same time point. Abbreviations: CF, cystic fibrosis; CFSPID, cystic fibrosis screen positive inconclusive diagnosis.

Legend:

------ chloride and conductivity cut-off for a intermediate test result

------ chloride and conductivity cut-off for a CF positive test result

____ estimated linear regression line

Figure 3. Bland-Altman plot of differences between sweat test conductivity from Nanoduct and chloride concentration from Macroduct vs. their averages, by final diagnosis (n=229). The Bland-Altman plot shows the difference of the sweat conductivity minus the sweat chloride on the y-axis, plotted against the mean of the conductivity and chloride value on the x-axis. This allows to identify proportional bias, i.e. whether the difference between the two tests is equal throughout the range of sweat test measurements. Abbreviations: CF, cystic fibrosis; CFSPID, cystic fibrosis screen positive inconclusive diagnosis.

Legend:

____ mean of difference ------- +/- 1.96 SD ____ estimated linear regression line

Figure 1. Proportion of succesful Macroduct and Nanoduct sweat tests by body weight and CF-centre

Figure 1A

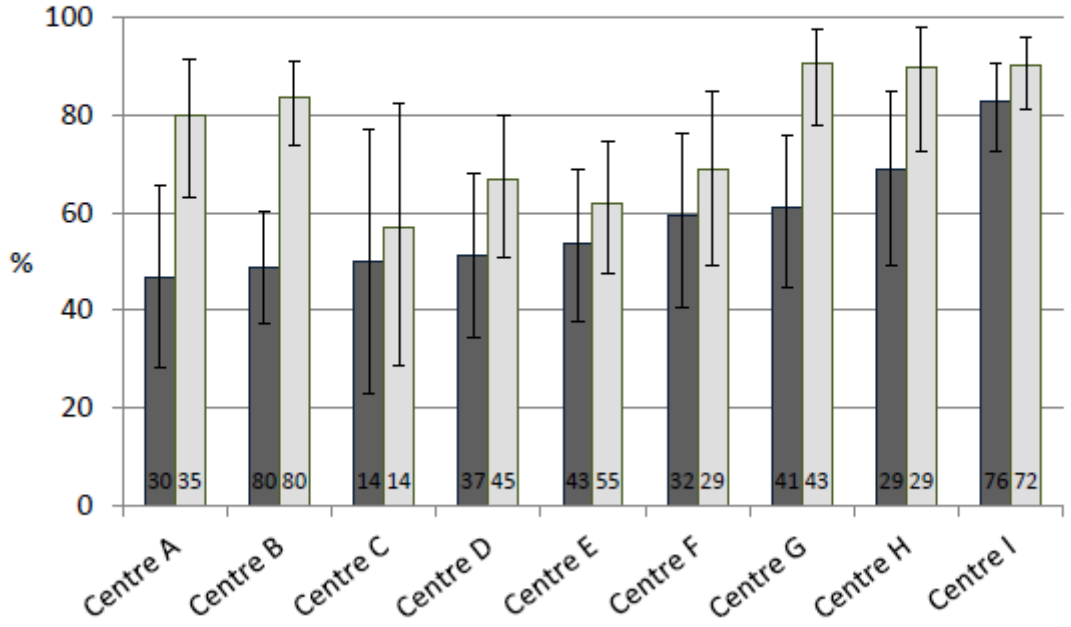

Figure 1B

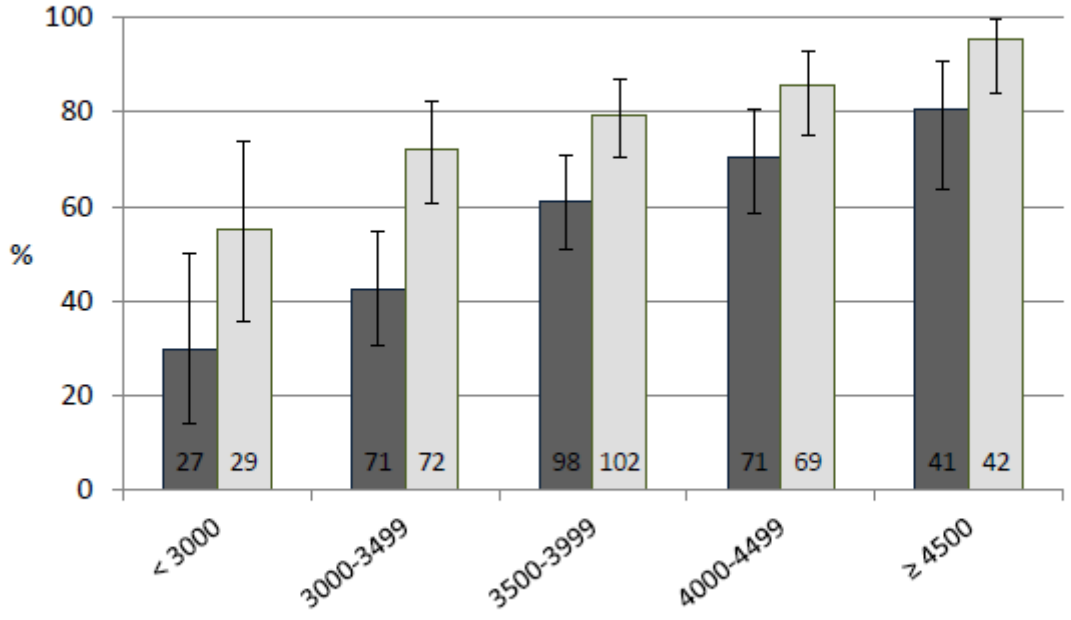

Figure 2. Scatterplot comparing Macroduct and Nanoduct test result, by final diagnosis (n=229)

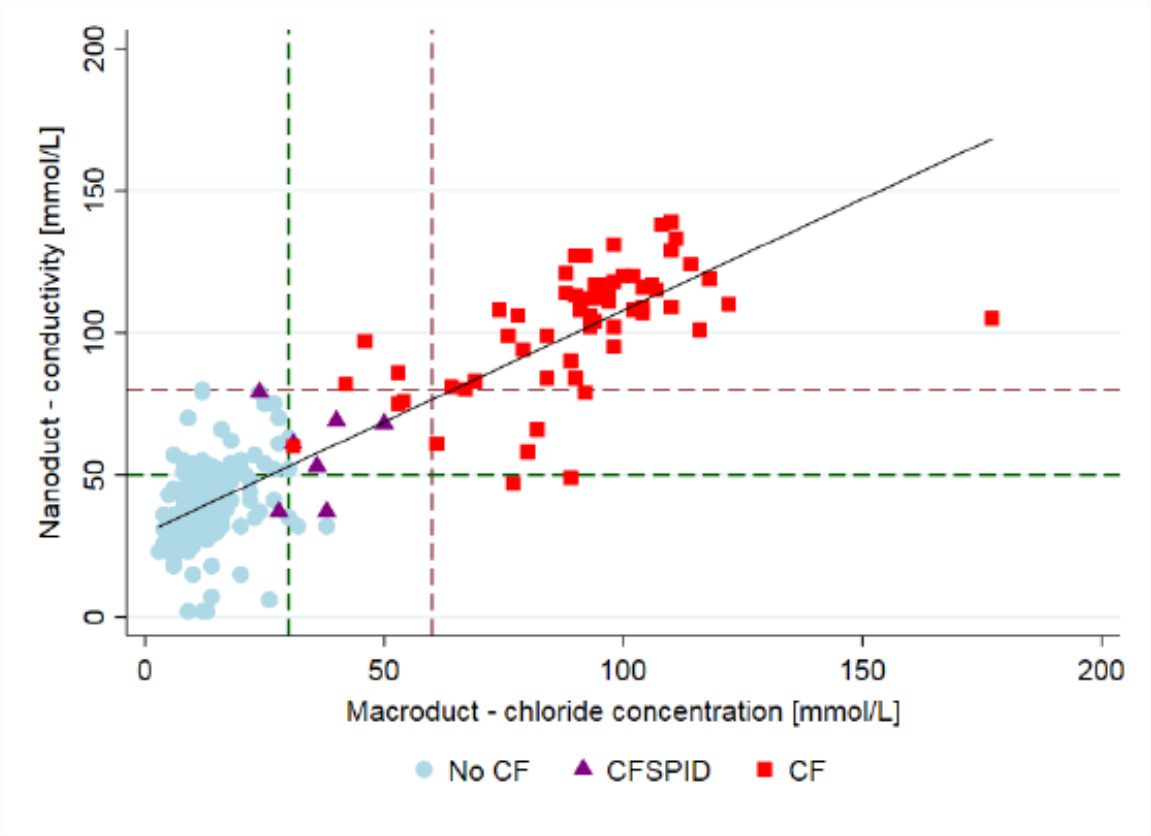

Figure 3. Bland-Altman plot of differences between sweat test conductivity from Nanoduct and chloride concentration from Macroduct vs. their averages, by final diagnosis (n=229)